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Biotechnology in Sweden

Claire Zomzely-Neurath

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<p>The enormous potential of biotechnology for wide-scale industrial application has been emphasized recently in Sweden by increased support by government and industry for basic and applied research. This report examines biotechnology research at the Royal Institute of Technology, Stockholm; the Lund Institute of Technology; and the Chalmers Institute of Technology, Göteborg. <i>Reynolds 2</i></p>			
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BIOTECHNOLOGY IN SWEDEN

1 INTRODUCTION

The enormous potential of biotechnology for wide-scale industrial application has been emphasized recently in Sweden by increased government funding of basic and applied research in biotechnology as well as by support from industry. The quality of the research in Sweden, both basic and applied, is excellent. This report deals with biotechnology research at institutes in Stockholm, Lund, and Göteborg.

2 THE ROYAL INSTITUTE OF TECHNOLOGY, STOCKHOLM

This institute started as a school of crafts in the early 19th century. It has gradually developed into a technical university and now has a staff of about 2000. There are 10 schools or divisions of engineering sciences at the institute (Table 1). Technology training and research in biotechnology are carried out in the School of Chemical Engineering, which includes 12 departments (Table 2). However, only the Department of Biochemistry and Biotechnology will be covered here. The research programs of this department are concentrated in six different areas of research (Table 3), and the format of the study program is shown in Figure 1. A summary of the present research projects at the Department of Biochemistry and Biotechnology is presented in Table 4. The research in the Department of Biochemistry and Biotechnology is innovative and contributes important data for the advancement of biotechnology. The variety of projects and the progress made by these scientists is extensive, especially considering that the staff is relatively small.

Since there are so many research projects in this department, only a few have been selected for a more detailed description.

Large-Scale Production of Chromatophores From *Rhodospirillum Rubrum* by Light-Fed Batch Cultivation

An important approach in enzyme technology is the use of stabilized sub-

cellular structures. One such example is chromatophores--i.e., membrane vesicles from photosynthetic bacteria with the capacity of cyclic photo-phosphorylation which can be used for adenosine triphosphate (ATP) regeneration without formation of by-products or consumption of auxiliary substances in the regeneration stage. The production costs, the stability, and the specific activity are of fundamental importance for the industrial use of such biocatalysts. On a small scale, the production of intracellular membrane (ICM), from which chromatophores can be prepared, has been thoroughly studied in order to investigate the mechanisms of the genesis of the ICM. For large-scale production, the conditions for light utilization are of special importance in order to obtain a high yield of cell mass, a high specific rate of ICM production, and a high overall ICM productivity.

To achieve large-scale production of chromatophores, A.L. Sneds and S.V. Enfors have cultivated the photosynthetic bacterium *R. rubrum* in a 50-L illuminated fermenter (pilot-plant scale). The influences of light intensity on the production of cell mass and the production of intracellular membrane, from which chromatophores can be prepared, were studied. They were able to obtain a high constant specific rate of intracellular membrane production by successively increasing the light intensity, i.e., by light-fed batch cultivation.

Control of Immobilized, Nongrowing Cells for Continuous Production of Metabolites

Immobilized viable cells can be either growing or nongrowing. One of the most distinguishing features of immobilized nongrowing cells is that the metabolic capacity of the cells can be used for a longer period of time. Part of the substrate which otherwise would have been used for biomass production is saved for product formation, which gives a higher overall yield of products and reduced biomass production. However, immobilized nongrowing cells lose activity with time.

C. Förberg, S.V. Enfors, and L. Häggström have recently devised a technique for maintaining constant activity during continuous production with immobilized nongrowing cells. A single-stage continuous system with alginate immobilized *Clostridium acetobutylicum* was mainly fed with a glucose medium that supported fermentation of acetone-butanol but did not permit microbial growth. The inactivation that occurred during these conditions was prevented by pulse-wise addition of nutrients to the reactor.

Using this technique, the researchers reduced the ratio of biomass to butanol to 2 percent (w/w), compared to 34 percent in a traditional batch culture. At steady-state conditions, butanol was the major end product, with a yield coefficient of 0.20 (g/g glucose). The production of butanol was 16.8/L/day during these conditions. In a corresponding system with immobilized growing cells, the ratio of biomass to butanol was 52 to 76 percent, and the formation of butyric and acetic acid increased, thereby reducing the yield coefficient for butanol to 0.11 (g/g). With the intermittent nutrient dosing technique, constant activity from immobilized cells was achieved for as long as 8 weeks. Thus the nutrient dosing technique described here offers two advantages: (1) it enables continuous production of metabolites from immobilized nongrowing cells for long periods of time, and (2) it offers a means to force the cells to grow mainly at the support surface, which improves the mass transfer and reduces the loss of substrate through cells leaving in the effluent.

Control of Cell Adhesion and Activity During Continuous Production of Acetone and Butanol With Adsorbed Cells

The following are important in immobilized cell systems intended for large-scale production: (1) factors like cost, availability, and stability of the immobilization material; (2) simplicity and mildness of the immobilization methods; and (3) ability to control and maintain the activity of such

systems. Adsorption represents a cheap, mild, and easy-to-scale-up immobilization method for which a variety of support methods are available. Moreover, the immobilization procedure can be carried out, in place, in the reactor to be used for production. The use of thin layers of adsorbed cells also improves the mass transfer situation over that of entrapped cells because of diffusion limitation in the latter case.

Adsorption is a mild and useful immobilization method when living cells are required for multistep reactions. Multiplying adsorbed cells will, however, result in a substantial cell leakage from the system. The use of nongrowing but viable cells reduces this disadvantage, although nongrowing cells lose activity with time.

Förberg and Häggström, using the nutrient dosing technique described above for maintaining constant activity of immobilized nongrowing cells, have developed a procedure for the continuous production of acetone and butanol from nongrowing *C. acetobutylicum* adsorbed onto beechwood shavings. Different adsorption procedures, influenced by the nutritional conditions in the reactor, were investigated. The cells were kept in a nongrowing but active state by the previously developed nutrient dosing technique, which also reduced the cell leakage. The system could be operated with maintained activity and very low leakage for several weeks.

Measurement of Hydrogen Evolution by Oxygen-Limited *E. coli* Using a Hydrogen-Sensitive Sensor

When designing large stirred bioreactors, it is essential to take into account that there will be some degree of inhomogeneity with regard to mass transfer in the broth. This will be especially pronounced when the broth is viscous or has non-Newtonian flow characteristics. Perhaps the most critical parameter in this context is the dissolved oxygen tension (DOT) of the broth. Where there are stagnant zones, the DOT may reach critically low values,

while in zones with better stirring, the DOT may remain at an acceptable level. This could result in what might be called "partial anaerobiosis." In large reactors where the mixing time is sometimes on the order of minutes, organisms are thus subjected to DOT which may oscillate with an amplitude and frequency characteristic of the specific bioreactor. This oscillation may be detrimental to the performance of the process.

If DOT measurement is used to monitor the situation, it is not sufficient to place a probe at one site in the reaction. To obtain a better representation of the DOT distribution, it must be measured at many sites in the bioreactor. Another way to detect the appearance of partial anaerobiosis is to analyze a metabolite excreted by the organism under conditions of anaerobiosis or low DOT and assay for it during the control of the fermentation. The appearance of this metabolite then indicates that a critically low DOT had been reached somewhere in the reactor.

N. Cleland, E.G. Kornstern, H. Elwing, S.O. Enfors, and I. Lundström have approached the problem described above by using a palladium metal oxide semiconductor-based (Pd-MOS) hydrogen gas sensor to detect molecular hydrogen, which is characteristically evolved by *E. coli* during mixed acid fermentation. Under conditions of oxygen limitation there was a sharply defined evolution of hydrogen gas which was reversible with respect to an increase in aeration. These researchers suggest that the sensor could be used for characterization of mixing in scale-up studies of bioreactors. Furthermore, one of the advantages of the use of hydrogen gas analysis in this context is that the semiconductor-based sensor is simply connected to the effluent gas stream of the reactor and that the sensor works continuously on line with response time on the order of seconds. Moreover, hydrogen is not substantially remetabolized under aerobic and anaerobic conditions, as are other metabolites.

Monitoring Glucose Consumption in an *E. coli* Cultivation With an Enzyme Electrode

Glucose is of such biological importance that much work has been done on developing methods for its detection and determination. Glucose electrodes based on enzymatic oxidation of glucose have been constructed, especially for clinical purposes. In the field of fermentation processes, however, relatively little work has been done. When an enzyme electrode is used in a fermentation process, serious difficulties arise. Sterility must be maintained (which means that the electrode must be autoclavable). In aerobic fermentation, the dissolved oxygen concentration in the broth may change from saturation to zero in a few hours, and the composition and ionic strength of the broth may undergo substantial changes. In anaerobic fermentations, the dissolved oxygen concentration is zero at all times.

There are two basic types of enzymatic analyzers. One is the enzyme reactor, in which sample is drawn from the sampling site of analysis, sometimes dialyzed, and pumped to an enzymatic sensor situated elsewhere. The other is the enzyme electrode which is self-contained and is placed directly in the sample. Each has inherent advantages and disadvantages. N. Cleland and S.O. Enfors have modified a previously developed autoclavable enzyme electrode, the externally buffered glucose oxidase electrode. This was used to monitor glucose consumption during batch cultivation of *E. coli*. The electrode signal showed good correlation with data from a conventional procedure and was independent of the dissolved oxygen in the fermentation broth. The latter feature was the main difference in behavior of the present electrode from the one used previously by this group.

Protein Purification Using Extraction in Aqueous Two-Phase Systems

Proteins are the product made available by the new developments in genetic engineering. Currently, *E. coli* is the main host organism for this

production, although efforts are being made to use other organisms. From the genetic point of view, however, *E. coli* is a superior host. The two main objections to this choice are: (1) the restrictions caused because the organism is not classified as "generally recognized as safe" in food legislation, as are *S. cerevisiae* and *B. subtilis*, and (2) *E. coli* does not excrete its proteins as does, for example, *B. subtilis*. If the technical problems involved in the large-scale isolation of intracellular proteins of *E. coli* can be solved, the genetic advantages of this organism could lead to greater industrial benefits.

One of the major problems in large-scale isolation of intracellular proteins is the separation of cell debris and DNA from the protein fraction. Another major problem is caused by proliferation of protease activity during cell disintegration. This phenomenon emphasizes the need for rapid operations in all steps--from disintegration to protease separation.

A. Veldi, T. Lindbäck, and S.O. Enfors have tackled the problem described above by using extraction in aqueous two-phase systems, an approach which overcomes many of the problems in the primary purification procedure. They have previously reported on a batch process for large-scale isolation of β -galactosidase from disintegrated *E. coli* cells. Recently, they have investigated the effects of biomass on their phase system and on the mass transfer of total protein, β -galactosidase, and DNA. Based on the data obtained from these studies, the researchers have developed a process for fast isolation of a β -galactosidase-rich protein fraction of *E. coli* from cell debris, DNA, and proteases by means of continuous extraction in an aqueous two-phase system. The basic composition of the phase system is polyethylene glycol (PEG) 4000, potassium phosphate, and water with a K_2HPO_4/KH_2PO_4 molar ratio of 1.42, giving the phase system a pH of 7. Phase system A was pure PEG and potassium phosphate, while B was the same but supplemented with 26.3 mM

tris-HCl. These researchers are now engaged in scaling up their procedure to production capacity.

Production of Small Polypeptides Using a Staphylococcal Protein A Vector

Protein engineering provides the means to alter the structure of a protein in a predictable fashion by genetic approaches, such as gene fusions and site-directed mutagenesis. For example, with a single point mutation in the cloned gene encoding the enzyme tyrosyl-tRNA (transfer RNA), a spectacular 100-fold increase in enzyme-substrate affinity has been achieved. Gene fusions have also been used to protect oligopeptides such as somatostatin and proinsulin from rapid proteolytic degradation when synthesized in *E. coli*. Easier purification of the desired product can also be achieved by facilitating secretion with specific leader sequences or by fusing affinity "tails" to the gene product. Using site-directed mutagenesis, it is also possible to introduce a specific chemical or enzymatic cleavage site at the junction between the two proteins.

M. Uhlen and his coworkers in the Department of Biochemistry and Biophysics have recently used a novel approach for the production of polypeptides using a staphylococcal protein A vector. The staphylococcal protein A gene is well suited for gene fusions because the fusion proteins produced can be efficiently purified by IgG (immunoglobulin G) chromatography with at least 95-percent yields obtained by Uhlen et al. In contrast to β -galactosidase fusions, the protein A hybrids can be translocated through the cytoplasmic membrane with the aid of a signal sequence. This may protect the heterologous protein from intracellular proteases and could also facilitate the correct formation of the disulfide bridges, which are rarely formed in the reducing environment of most bacterial cytoplasms.

Uhlen et al. have recently been able to produce human insulin-like growth factor I (IGF-I) using a protein A gene fusion vector. IGF-I is a

70-amino-acid, single-chain polypeptide with growth-promoting effects *in vitro* and *in vivo*. It belongs to a subgroup of growth factors called somatomedins, which are thought to mediate the growth-promoting effects of growth hormone (GH). Early attempts to express a synthetic gene encoding the mature IGF-I in *E. coli* gave low levels of production which provided the impetus for Uhlen et al. to develop alternative host-vector systems. Using the Gram positive host *Staphylococcus aureus*, Uhlen et al. were able to achieve expression and secretion of a fusion protein consisting of protein A and IGF-I. This fusion protein is recovered in high yield by passing the culture medium through an IgG affinity column. Using site-specific mutagenesis, the researchers introduced an acid labile aspartic-proline cleavage site at the fusion point between the two proteins. The protein A "tail" was then removed from the affinity-purified fusion protein by chemical cleavage, releasing biologically active IGF-I molecules. Thus, Uhlen et al. have shown that the staphylococcal protein A gene fusion system can be used efficiently to express, secrete, and purify foreign gene products. Furthermore, Uhlen et al. have found that the expression from the protein A promoter can be varied 100-fold by selecting different strains of *S. aureus*. Also, a 20-fold increase in yield can be obtained simply by growing the cells in high amounts of streptomycin. This expression vector system developed by Uhlen et al. is of value for the production and purification of foreign gene products, regardless of whether the protein will be used for immunization or for structural studies.

3 LUND INSTITUTE OF TECHNOLOGY, UNIVERSITY OF LUND

Researchers at this institute are carrying out a wide variety of projects in the area of biotechnology, as shown in Table 5. These scientists are making important contributions to research in biotechnology with a high potential for eventual industrial application. A major

center for applied biotechnology research is being built adjacent to the Lund Institute of Technology. This center will receive support from both the Swedish government and industry. The purpose of the center is to use more efficiently the results of the research at the Lund Institute of Technology in direct application to industrial problems. The scientists at the center will be a mixed group--i.e., some employed by industry, others by the university.

A few of the research projects which have direct or potential significance to industrial application are described below.

Immobilized Cells

Immobilized whole cells entrapped in polymeric matrices have, during recent years, been studied intensively for biotechnological applications. In most instances a single enzymatic activity present in nonviable cells has been used. The potential usefulness of immobilized cells in order to carry out complex biochemical conversions and syntheses is great, but usually requires intact and viable cells. The lack of suitable techniques with which to immobilize whole cells while preserving their viability has, to some extent, hampered their use.

Optimal immobilized-catalyst preparations usually require spherical particles because they are homogenous and facilitate column packing. In the past, spherical particles of some polymers containing entrapped enzymes have been made by carrying out gel formation in an organic phase such as toluene:chloroform, which, however, does not leave entrapped cells in a viable state. However, K. Mosbach and his coworkers in the Department of Pure and Applied Biochemistry have developed a general method for the immobilization of cells with preserved viability. The method, which can be used for a wide variety of polymers, is based on a two-phase system in which the hydrophobic phase is composed of an inert liquid such as vegetable oil or tri-n-butylphosphate. The cell polymer/monomer mixture is kept

suspended in a hydrophobic phase which is compatible with the cells. Various monomers or polymers were tested, including agarose, agar, carrageenan, alginate, fibrin, and polyacrylamide. Furthermore, by adjustment of the stirring speed of the suspension, beads of a desired diameter can easily be obtained. The entrapped cells are fully viable and biosynthetically active. Mosbach et al. have successfully immobilized microbial, algae, plant, and animal cells using their method.

P. Adlercreutz and B. Mattiasson have been studying the problem of oxygen supply to immobilized cells. Oxygen supply is a critical point in technical processes when aerobic cells are used in immobilized preparations. One reason is the low solubility of oxygen in water solutions. If the cells are immobilized before bioconversion takes place, the problems concerning oxygen supply become even more severe. This is due to the extra resistance to oxygen transport: oxygen must diffuse through the matrix to reach the cells.

Several methods have been tried by these researchers to increase the oxygen supply to immobilized biocatalysts. Oxygen has been added as hydrogen peroxide, which is decomposed by catalase in the reactor. Another way to generate oxygen in the reactor is to coimmobilize the oxygen-consuming organism with oxygen-producing organisms--for example, algae. The water solutions normally used can sometimes be replaced by organic solvents which dissolve more oxygen. Adlercreutz and Mattiasson have also used other ways to increase the oxygen content of the medium, such as the addition of hemoglobin or emulsions of petrochemicals.

Recently, these investigators have used an entirely different approach. They used p-benzoquinone as a substitute for oxygen in the oxidation of glycerol to dihydroxyacetone by immobilized *Gluconobacter oxydans* cells. The reaction rate was much higher when p-benzoquinone was used than when oxygen was used. In an experiment with free cells, p-benzoquinone gave a rate more than four times

that of oxygen, and with immobilized cells the difference was even greater. The p-benzoquinone is more effective than oxygen because it gives a higher maximal reaction rate and because it is more soluble in water than oxygen. The operational stability of the process was much better than when oxygen was used. Also, the by-product formed from p-benzoquinone--i.e., hydroquinone--can be oxidized to p-benzoquinone which can be reused. These researchers carried out seven successive regenerations of p-benzoquinone without any loss of efficiency. They consider that p-benzoquinone and similar artificial electron acceptors can be used to increase the efficiency of many bioconversions similar to the oxidation of glycerol to dihydroxyacetone, an example of a microbial oxidation reaction.

Biosensors

In many studies it has been shown that analysis of ethanol and other volatile organic compounds can be performed with gas-sensitive detectors in combination with gas-permeable membranes. Flame ionization detectors or semiconductor gas sensors have usually been used for detection. The membranes most frequently used have been porous teflon or silicone tubing. All of the analyzers used in the past lose sensitivity at higher concentrations due to the saturation of the gas detector. To economically scale up fermentative production of ethanol and acetone/butanol, one must accurately measure higher concentrations of these compounds. Thus, ethanol concentrations in fermentations must be followed up to and above 7 percent. The nonlinearity in this concentration region in previous methods has restricted the use of gas sensors when monitoring fermentation processes. One way to make these sensors more versatile is to incorporate a continuous dilution system into the device, thereby making it possible to dilute either the medium to be analyzed, or the permeated gases, or both, before the analysis. Mandenius and Mattiasson have applied two such systems primarily to the analysis of

ethanol, but also to butanol and acetone, formate, acetate, and formaldehyde. These investigators designed: (1) a gas dilution system, and (2) another system in which the dilution was accomplished by diluting the medium before it was exposed to the membrane. These two systems for sample treatment of volatile organic compounds in fermentation media were combined with silicone gas sensors for continuous on-line analysis. By using continuous dilution of liquid or gas flow streams, the low limit of detection for ordinary gas detectors was extended to operate within concentrations ranges of importance in biotechnological processes. The systems are inexpensive compared with others. The equipment can be sterilized *in situ*, is simple to handle, and is suitable for continuous day and night operation, primarily because there is little need for maintenance. Furthermore, in all cases, the performance of both systems was maintained for long periods of time.

C.F. Mandenius, L. Bülow, B. Danielsson, and K. Mosbach have recently developed a greatly improved method for the monitoring and control of enzymatic sucrose hydrolysis using on-line biosensors.

On-line monitoring and on-line control are two tightly connected concepts. On-line process control without on-line sensors is frequently performed today, but can usually be significantly improved when combined with on-line monitoring of appropriate process parameters. However, the development of biosensors capable of measuring substrates and products of fermentation or an enzymic conversion is only of recent date. Among such biosensors are enzyme electrodes and enzyme thermistors (enzyme-based flow calorimeters). Using these devices, researchers have analyzed glucose and sucrose by applying the enzymes glucose oxidase and invertase, respectively. These biosensors have also been tested for on-line applications by Mosbach et al. In order to be applied in an industrial process, these biosensors have to be combined with systems for sample treatment for two pri-

mary reasons. Enzymes usually do not operate in the concentrations normally present in industrial processes, which makes it necessary to dilute the samples continuously. Secondly, such samples often contain impurities that may either negatively affect the enzymes by denaturation or invoke clogging/fouling of membranes or filters. These problems are relevant regardless of whether the analytical instrument is used in flow systems.

Mosbach et al. are using a biosensor, the enzyme thermistor (flow microcalorimeter containing immobilized enzymes), to monitor the substrate and product concentrations from an invertase reactor, while controlling the conversion by changing the influent feed concentration of sucrose. A microcomputer is used to control an automatic sampling system comprising intermittent flow injection and peak height evaluation. These evaluations are used in an algorithm routine which controls the speed of the influent pumps. Both the glucose and sucrose signals are used for the control. Based on their studies, these investigators state that: (1) it is possible to use the computerized monitoring system for regulation purposes using simple control algorithms; (2) enzyme thermistor devices can be combined with computers for evaluation and sampling procedures; (3) the heat recording capacity of the enzyme thermistor device is accurate enough to resolve small and slow changes in concentration of the type that is likely to appear in large-scale reactors; and (4) it is possible to extend the small linear range for on-line glucose determination to include concentrations prevalent in industrial application scales. The system described by Mosbach et al. could be extended with other thermistor sensors for glucose and sucrose determination. A combination with glucose and sucrose measurements in both the influent and effluent of the hydrolysis reactor would also provide the means for indirect evaluation of fructose. Furthermore, a corresponding on-line system would provide monitoring and control facilities

for production of high-fructose syrup from sucrose rich feedstocks.

C.F. Mandenius, B. Danielsson, and K. Mosbach have developed another method for continuous sampling from complex solutions--such as fermentation broth, milk, or wastewater--to yield samples suitable for liquid chromatography, flow injection analysis, enzyme calorimetry, etc. They are using a dialysis probe which they designed for continuous sampling in fermenters and in complex media. In their procedure, the analyte is transferred to a flow stream separated from the sample by a dialysis membrane that is protected from fouling by a strong tangential flow of the sample solution. This flow is accomplished by placing a magnetic stirring bar close to the membrane surface. The device is constructed of materials permitting the probe to be steam-sterilized when mounted inside a fermenter. In their design, a plastic probe is covered by dialysis tubing. The probe is inserted into the test solution, and the permeated solution is recovered through a spiral groove machined around the surface of the probe. Mandenius et al. have obtained a patent for their dialysis probe.

Binding of Biomolecules to Silicone Surfaces

C.F. Mandenius, S. Welin, B. Danielsson, I. Lundström, and K. Mosbach have developed two methods for the chemical binding of biomolecules to silicone surfaces. The first method uses an alkyl silane and a nucleophilic reagent to join the biomolecule to the silicone surface; the second method involves cross-linking with glutaraldehydes in order to couple the biomolecule and albumin molecules, which first have been physically adsorbed. The course of binding to the silicone surface has been followed with the aid of ellipsometry. This optical measuring technique estimates the thickness of--for example, organic layers--by measuring the polarization properties of a light beam before and after reflection at surfaces. These investigators studied the following sys-

tems: (1) concanavalin A- *S. cerevisiae* cells, immunoglobulin G- *S. aureus* cells, and an NAD-analog-lactate dehydrogenase. With ellipsometry, it was possible to assess how the results were influenced by the incubation time, the concentration of the cells, and the biomolecules added. They found that an increasing time of incubation and higher concentration resulted in a more complete coverage of the silicone wafer surface. This method is very useful for molecular studies of biomolecules such as monoclonal antibodies, viruses, or other molecules. In addition, comparative information can be obtained with respect to the kinetics of binding and to concentration dependence.

Enzyme Purification

A new enzyme purification method, affinity precipitation, has been developed by S. Flygare, T. Griffin, P.O. Larsson, and K. Mosbach. Affinity precipitation is a novel technique closely related to immunoprecipitation and affinity chromatography. The first step in affinity precipitation involves the mixing of a bifunctional ligand with an oligomeric enzyme. A precipitation occurs if the spacer linking the two ligand entities together is long enough to bridge the distance between the two enzyme molecules, and if the binding between the ligand and enzyme is strong enough. This reaction takes place because a bis-ligand interacts simultaneously with two enzyme molecules. Since the enzyme is oligomeric, it was found that an elaborate network of enzymes and bifunctional molecules forms. When such a network grows to sufficient size, it no longer remains in solution but precipitates out. These investigators found that to improve further the selectivity of the procedure and to increase the effective binding between ligand and enzymes, ternary complex formation could be used. For example, a BIS-NAD analog together with pyruvate forms a strong complex with the active site of soluble lactate dehydrogenase, enabling the enzyme to precipitate. Flygare et al. achieved affinity precipitation of

several dehydrogenases with NAD derivatives. They also carried out affinity precipitation on a preparative scale for the isolation of heart lactate dehydrogenases from a crude extract obtaining good yields and purity of the enzyme.

4 CHALMERS INSTITUTE OF TECHNOLOGY, GÖTEBORG

This institute is a division of the University of Göteborg and is therefore supported by the Swedish government. At this time, extensive reorganization of the institute is taking place and will include a change in emphasis of research projects as well as closer collaboration with industry. In the past, the Chalmers Institute of Technology has been concerned primarily with teaching, with minor emphasis on advanced training and research, compared with the technology institutes in Stockholm and Lund. Future plans are to develop programs with a major emphasis on research projects in biotechnology and graduate training similar to the programs at the other two institutes.

B.G. Malmström and his group have been engaged in research on cytochrome oxidase and related enzymes, lipoxygenase, ribulose-1-5-bisphosphate carboxylase/oxygenase mechanism, and electron transport chains in photosynthetic systems. They have carried out very detailed studies in these areas and have made important contributions.

There is also a very good, but small, recombinant DNA (rDNA) group at this institute. Future plans include

increased funding and personnel for the rDNA work. For example, L.G. Lundberg, C.H. Karlström, P.O. Nyman, and J. Neuhaard have recently constructed thermoinducible plasmids carrying the gene (*dut*) for the enzyme, deoxyuridine 5'-triphosphate nucleotidohydrolase (*duTPase*) from *E. coli*. These researchers have studied expression of the *dut* gene of *E. coli* using two fundamentally different types of thermoinducible vehicles: (1) plasmids with runaway replication at high temperature, and (2) a plasmid with a promoter whose expression is induced at high temperature. The *dut* gene of *E. coli* has been cloned in the thermoinducible plasmids, isolated, and characterized. Bacterial strains carrying the plasmid pkk 150 (derived from pkk 2164 by an insertion of an *ECO* RI+*Sac* I restriction enzyme fragment from phase λ) obtained by Lundberg et al. should be useful for the preparation of *duTPase* in large amounts for structural and functional studies since about 10 percent of total cell protein after thermoinduction was found to be *duTPase*. The pkk 150 plasmid can also be useful for cloning of other genes using the *Sac* I site.

5 CONCLUSION

Biotechnology research at the institutes of technology in Stockholm, Lund, and Göteborg--especially the first two institutes--is innovative and has resulted in important contributions with present and potential applications in industry.

Table 1

**Royal Institute of Technology,
Stockholm Sweden**

School of Engineering Physics
School of Computer Science and Engineering
School of Vehicle Engineering
School of Electrical Engineering
School of Civil Engineering
School of Chemical Engineering
School of Metallurgy and Materials Technology
School of Architecture
School of Surveying

Each year 1000 students initiate their 4-year training program specializing in one of the 10 engineering sciences according to the schools listed above. An additional 1000 students are engaged in higher studies. The time to obtain the Doctorate of Technology is about 5 years, and a Licentiate of Technology takes about 2½ years. There are 110 professors guiding research and education at the Royal Institute of Technology.

Table 2

**The School of Chemical Engineering,
Royal Institute of Technology**

This school includes the following 12 departments:

Analytical Chemistry
Biochemistry and Biotechnology
Cellulose Technology
Physical Chemistry
Chemical Engineering
Chemical Technology
Nuclear Chemistry
Inorganic Chemistry
Organic Chemistry
Paper Technology
Polymer Technology
Applied Electrochemistry and Corrosion Science

120 students per year are admitted for basic training aimed at a degree in chemical engineering specialized in one of five areas: chemical engineering, chemistry, polymer technology, cellulose and paper technology, and biotechnology. The permanent staff is 500, including 17 professors. About 260 postgraduate students are registered for higher studies, some of them doing their research work in industry.

Table 3

**The Department of Biochemistry and Biotechnology,
Royal Institute of Technology**

The research program of the department is concentrated in the following areas:

Microbial metabolism
Recombinant DNA (rDNA) technology
Bioorganic synthesis
Plant tissue culture
Fermentation technology
Extraction of extracellular enzymes

The department has a staff of about 35, including professors of biochemistry (Sten Gatenbeck) and biotechnology (Sven Olaf Enfors), and an adjunct professor in wastewater treatment (Jan Rennersfelt). There are 22 doctoral students, and each year about 25 students specialize in biotechnology to obtain their chemical engineering degree. The courses that specialize in biotechnology are mainly during the last year of the 4-year program (Figure 1). The facilities include, in addition to laboratories and offices, a pilot plant for scaling up of fermentation and downstream processing to 1-m³ fermentation scale.

Table 4

Current Research Projects at the Department of Biochemistry and
Biotechnology, Royal Institute of Technology

Microbial Metabolism

(1) Metabolic pathways and regulation of intermediary metabolism and key enzymes in solvent-producing Clostridia. Researchers: M. Hartmanis, S. Gatenbeck. (2) Mycotoxins, their biosynthesis and metabolism on cereals. The project is focused on the nephrotic agent ochratoxin A, its occurrence in nature (cereals and animal blood), and its metabolism in animals. Researchers: K. Hult, S. Gatenbeck. (3) Continuous cultivation of yeast in phenol-chemostat. The aim is to develop a technique for continuous fermentation of yeast strains with the ability of growing on phenols. (4) Reaction mechanisms of phenolhydroxylases. (5) Photochemical energy transport via hydrogenase. Researchers: M. Mörtberg, A. Spanning, T. Sejlitz, and H. Neujahr.

Recombinant DNA Technology

(1) Construction of gene fusion vectors based on the gene for staphylococcal protein A. By this construction, proteins can be purified in a one-step procedure by affinity chromatography. The system has already been shown to work for β -galactosidase and for a human growth hormone. (2) Construction of monovalent protein A by *in vitro* techniques. A gene is being constructed coding for only one IgG-binding domain of protein A. This is done by exonuclease treatment of the whole gene. (3) Gene fusion vectors based on the signal sequence of the gene for protein A. This work deals with fusing genes coding for different proteins to the signal sequence originally from protein A. The purpose is to study protein secretion in Bacillus and Staphylococcus. (4) Isolation of the gene for penicillin V amidase. The gene for a prokaryotic penicillin V amidase has been isolated and amplified. The nucleotide sequence is now being determined. (5) Isolation of the genes for biotin biosynthesis in Bacillus. The genes for the biosynthesis of biotin from a Bacillus species is being isolated. The purpose is to amplify the gene product and by a suitable precursor to overproduce biotin. (6) Cloning systems in Streptomyces. Genes are introduced into Streptomyces to study the genetics of the biosynthesis of D-Cycloserin. A sequencing system for transcriptional promoters from Streptomyces is being set up. Researchers: M. Uhlen, B. Nilsson, A. Olsson, T. Moks, M. Eliasson, G. Emås, T. Hagström, L. Abrahmsén, and S. Gatenbeck.

Biorganic Synthesis

Biorganic synthesis of chiral synthons of industrial and pharmaceutical interest. In this project, the enantioselective hydrolysis of prochiral esters is being studied. Researchers: J. Briteje, K. Hult, and S. Gatenbeck in collaboration with the Department of Organic Chemistry.

Plant Tissue Cultures

(1) Design, function, and degradation of fungicides. (2) Production of secondary metabolites by plant cell and tissue cultures. (3) Degradation of enzymes in plants. Researchers: L. Björk and A. Ohlsson.

Fermentation Technology

(1) Sensors and computer strategy for process control. Enzyme electrodes for on-line analysis and fed batch control in E. coli fermentation. A system for fermentation control by means of a computer with standard functions (stirring, temperature, etc.) controlled by an Assembler program with biological and mass balance parameters measured and evaluated by a basic program. Researchers: N. Cleland, A. Larsson, and S.O. Enfors. (2) Adsorbed nongrowing cells for continuous production of metabolites in aerobic processes. A nutrient dosing technique for maintaining constant activity in immobilized nongrowing cells has been developed. Current work is focused on development of adsorption techniques, reactor construction, mass transfer problems, and metabolic regulation in amino acid production from E. coli. Researchers: C. Förberg and L. Häggström. (3) Biological responses to mixing during scale up of aerobic processes. The biological response to short time interruption of aeration is measured with various techniques. Kinetics of inhibition of Pc. chrysogenum is being investigated. The response of

Table 4 (Cont'd)

E. coli to oxygen starvation is analyzed by means of a special hydrogen sensor in the exit gas. Researchers: N. Cleland, G. Larsson, and S.O. Enfors. (4) Scale up of recombinant DNA (rDNA) processes. Processes with rDNA organisms produced by the rDNA group of the department are studied in scale-up experiments in the pilot plant with respect to fermentation and a separation of the products. Currently being studied are: (a) production of the fused proteins E. coli- β galactosidase-Staphylococcus aureus-protein A produced by E. coli, and (b) S. aureus Protein A-human IGF-1 produced by S. aureus. Researchers: L. Strandberg, M. Nettelbladt, B. Nilsson, and S.O. Enfors. (5) Properties and principles of mycelial flow. This project deals with the rheology and flow mechanisms in a mycelial mash. Mathematical modeling is used for interpretation of experimental results. Researcher: U. Björkman

Separation Technology

(1) Continuous extraction of β -galactosidase from E. coli. Effects of biomass load on mass transport and partitioning of cell disintegrate components in polyethylene glycol-phosphate two-phase systems have been studied and a 1-m³ scale process for the enzyme production has been developed. (2) Extraction of membrane-bound enzymes in aqueous two-phase systems. Chromatophores in aqueous two-phase systems from Rhodospirillum rubrum are produced and clarified by extraction. The ATP-regenerating capacity of the chromatophores is being studied. (3) Affinity extraction of proteins. Various methods for applying affinity techniques to adsorb the protein products at an early event during the process are being studied for the purpose of scaling up the process. Application of the β -galactosidase-protein A hybrid molecule produced by an E. coli developed by the rDNA group is one example of an affinity ligand that can be used for protein purification. Researchers: T. Lindbäck, A.L. Sneds, A. Veide, and S.O. Enfors.

Table 5

Pure and Applied Biochemistry: Lund
Institute of Technology, University of Lund, Sweden,
Research Division

1. Enzyme technology
 - Affinity techniques
 - Biosensors
 - Organic polymers with biological activity
2. Cell technology
 - Plant cells--alkaloids, drugs
 - Animal cells--interferon, monoclonal antibodies
 - Bacteria--steroids, vitamins, interferon, etc.
 - Yeasts--ethanol production
 - Fungi--antibiotics
3. Immunotechnology
 - Purification
 - Immunotherapy
 - New analytical systems
 - Lectins--host/parasite interactions
4. Gene technology
 - Immobilized restriction enzymes
 - Immobilized ligases
 - Product enhancement
5. Biochemical process technology
 - Process control--biosensors, computer assistance
 - Production/bioconversion--immobilized cells, two-phase systems, membrane reactors, oxygenation techniques
 - Downstream processing--membrane technology, affinity techniques

Year 1-3 Basic Chemistry and Engineering courses

Year 3 Organic Chemistry, a.c.*

Chemical Engineering, a.c.
Transport Phenomena, a.c.

Year 4 Biology (Lectures 21 hours)
Biochemistry, b.c.* (Lectures 56 hours,
Laboratory work, 98 hours)

Microbiology, b.c.
(Lectures 28 hours,
Laboratory work, 56 hours)

Analytical Chemistry, a.c.
Medical Technology
Clinical Chemistry

Biochemistry, a.c.**
(Lectures 14 hours)

Biotechnology
(Lectures 56 hours,
Laboratory work, 4 weeks)

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- ★ b.c.= basic course
- a.c.= advanced course
- ★ Mainly genetic engineering

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